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The Patent Office

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1. Your reference AP7

2. Patent application number *(The Patent Office will fill in this part)* **9903404.3** 16 FEB 1999

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Angiogene Pharmaceuticals Ltd
14 Plowden Park
Aston Rowant
Watlington OXON OX9 5SX

Patents ADP number *(if you know it)* 72444780 01

If the applicant is a corporate body, give the country/state of its incorporation Incorporated in Scotland

4. Title of the invention

Methods of treatment and compositions useful for the treatment of diseases involving angiogenesis.

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

10 Aston Park
Aston Rowant
Watlington OXON OX9 5SW

Patents ADP number *(if you know it)* 72444780 02

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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a) any applicant named in part 3 is not an inventor, or
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See note (d))

Yes

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Claim(s)	
Abstract	
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11. I/We request the grant of ~~a~~ patent on the basis of this application.

Signature [signature] Date 15/2/99

12. Name and daytime telephone number of person to contact in the United Kingdom Peter Davis 01844 354562

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METHODS OF TREATMENT AND COMPOSITIONS USEFUL FOR THE TREATMENT OF DISEASES INVOLVING ANGIOGENESIS.

This invention relates to a method for treating diseases involving active angiogenesis, to compositions useful for the treatment of diseases involving angiogenesis and to the use of the compositions in the preparation of a medicament for the treatment of diseases involving active angiogenesis. In one aspect of the invention the method involves the administration to a mammal of an inhibitor of nitric oxide in combination with a compound inducing vascular damage.

Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J Folkman, New England Journal of Medicine 333, 1757-1763, 1995). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy. In all these diseases reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect.

Certain chemical compounds have been shown to have vascular damaging activity against the newly formed endothelium of solid tumours. These agents include, for example, combretastatin A4 phosphate (Dark et al., Cancer Research 57, 1829-1834, 1997), combretastain analogues (for example those described in J Med Chem 41, 3022-32,1998 by Ohsumi et al.), the flavone acetic acids, for example 5,6-dimethylxanthenone acetic acid (Zwi, Pathology, 26, 161-9, 1994), colchicine (Baguley et al. Eur J Cancer 27, 482-7, 1991). However some tumours are resistant to these agents.

One characteristic of tumours relatively resistant to vascular damaging agents is their ability to produce large amounts of nitric oxide. The role of nitric oxide in tumour growth is unclear and there have been reports of both tumour-stimulating and tumour-inhibiting effects (Chinje and Stratford, Essays Biochem. 32, 61-72, 1997). It has been suggested that the antitumour effects of 5,6-dimethylxanthenone acetic acid are mediated in part by nitric oxide production (Thompsen et al. Cancer Chemother Pharmacol. 31,151-5, 1992).

We have found that the efficacy of vascular damaging agents can be improved by combination with inhibitors of nitric oxide synthases, the enzymes which produce nitric oxide from arginine. In particular the efficacy of vascular damaging agents against tumours relatively resistant to their effects is improved by treatment with a nitric oxide synthase inhibitor.

Accordingly in one aspect of the invention we provide a method of treatment for a mammal having a disease which involves active angiogenesis such method comprising the administration of a therapeutic or subtherapeutic amount of a vascular damaging agent together with an inhibitor of nitric oxide synthase in an amount sufficient to augment the effect of the vascular damaging agent. The method is useful

for the treatment of diseases such as cancers, especially solid tumours, psoriasis, diabetic retinopathy, macular degeneration, atherosclerosis and rheumatoid arthritis.

The vascular damaging agent and the nitric oxide synthase inhibitor can be administered together or separately. The method may be used as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide; antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example aspariginase; topoisomerase inhibitors for example etoposide, topotecan and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

The vascular damaging agent and the nitric oxide synthase inhibitor can be administered by the same route or by different routes. Such routes of administration include oral, buccal, nasal, topical, rectal and parenteral administration. Each component of the method, the vascular damaging agent and the nitric oxide synthase inhibitor may independently be administered in a form suitable for the intended route of administration and such forms may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion. The preferred route of administration of each component will depend on the disease being treated. For solid tumours the components may each advantageously be delivered, either together or separately, as an intravenous infusion.

Vascular damaging agents are those compounds known in the art which induce selective damage to newly formed, rather than established, vasculature. Such agents include tubulin-binding agents, for example the combretastatins and their prodrugs, the colchinols and their prodrugs and (Z)-2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenylamine and its prodrugs, TNF-alpha inducing agents such as the xanthenone acetic acids, for example dimethylxanthenoneacetic acid, and antibodies targeted to the vasculature.

Nitric oxide synthase inhibitors are those compounds known in the art which inhibit any of the forms of nitric oxide synthase. Such agents include derivatives of arginine, ornithine, lysine and citrulline, S-alkylthioureas and aminoguanidines. Where the nitric oxide synthase inhibitor is a derivative of arginine it may be, for example, an N^G-substituted L-arginine selected from N^G-nitro-L-arginine and alkyl esters thereof, N^G-methyl-L-arginine and N^G-amino-L-arginine. Where the nitric oxide synthase

inhibitor is a derivative of ornithine it may be, for example L-N6-(1-iminoethyl)-ornithine. Where the nitric oxide synthase inhibitor is a derivative of lysine it may be, for example L-N6-(1-iminoethyl)-lysine. Where the nitric oxide synthase inhibitor is a derivative of citrulline it may be, for example L-thiocitrulline, L-homothiocitrulline or an S-alkylthiocitrulline such as S-methyl-L-thiocitrulline.

In a further embodiment of the invention there is provided a composition for the treatment of diseases involving active angiogenesis. The composition of the invention comprises a vascular damaging agent in combination with a nitric oxide synthase inhibitor where both the vascular damaging agent and the nitric oxide synthase inhibitor are as hereinbefore defined.

Thus for example the composition may contain for example a combretastatin derivative, a colchicine derivative, a colchinol derivative, a xanthenone acetic acid derivative or a vascular targeted antibody, in combination with a nitric oxide synthase inhibitor for example a derivative of arginine, a derivative of ornithine, a derivative of lysine, a derivative of citrulline, a S-alkylthioureas or an aminoguanidine.

Particular examples of vascular damaging agents that may be present in the composition include combretastatin A4 and its prodrugs for example combretastatin A4 phosphate, (Z)-2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenylamine and its prodrugs, N-acetylcolchinol and its prodrugs for example N-acetylcolchinol-O-phosphate and 5,6-dimethylxanthenoneacetic acid.

Particular examples of nitric oxide synthase inhibitors which may be present in the composition include derivatives of arginine, ornithine, lysine and citrulline, S-alkylthioureas and aminoguanidines. Where the nitric oxide synthase inhibitor is a derivative of arginine it may be, for example, an N^G-substituted L-arginine selected from N^G-nitro-L-arginine and alkyl esters thereof, N^G-methyl-L-arginine and N^G-amino-L-arginine. Where the nitric oxide synthase inhibitor is a derivative of ornithine it may be, for example L-N6-(1-iminoethyl)-ornithine. Where the nitric oxide synthase inhibitor is a derivative of lysine it may be, for example L-N6-(1-iminoethyl)-lysine. Where the nitric oxide synthase inhibitor is a derivative of citrulline it may be, for example L-thiocitrulline, L-homothiocitrulline or an S-alkylthiocitrulline such as S-methyl-L-thiocitrulline.

The composition is useful for the treatment of diseases involving active angiogenesis for example solid tumours, psoriasis, diabetic retinopathy, macular degeneration, atherosclerosis and rheumatoid arthritis.

The relative proportion of each component will be determined by the identity of each individual vascular damaging agent or nitric oxide synthase inhibitor and by the disease to be treated.

The composition may include pharmaceutically acceptable excipients selected with regard to the intended route of administration and standard pharmaceutical practice. The composition may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the composition may

take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the identity of the individual components, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician.

The composition of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours the composition may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide; antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example aspariginase; topoisomerase inhibitors for example etoposide, topotecan and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

In a further embodiment of the invention we provide the use of a compostion of the invention for the preparation of a medicament for the treatment of a disease involving active angiogenesis.

The following biological assay can be used to illustrate the invention:

Activity against tumour vasculature measured by fluorescent dye.

Tumour functional vascular volume in Sarcoma S tumour-bearing mice (Parkins Cancer Res. 55, 6026-9, 1995) was measured using the fluorescent dye 3,3'-diheptyloxacarbocyanine (Molecular Probes Inc. Eugene, OR, USA). Five animals were used in control and treated groups. The fluorescent dye was dissolved in 3:1 dimethylsulphoxide:saline to a final concentration of 0.6mg/ml and injected intravenously at a dose of 1mg/kg body weight 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10 µm sections were cut at 3 different levels and observed under UV illumination. Blood vessels were identified by their fluorescent outlines using 490nm excitation with a 520nm filter and vascular volume was quantified using a point scoring system based on that described by Chalkley, (J Natl Cancer Inst, **4**, 47-53, 1943). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.

In this assay the effect of a given dose of either a vascular damaging agent or a nitric oxide synthase inhibitor administered alone can be compared with the effect of a combination of the two agents.

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Agent : Laughton Perry